

Appendix B
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1. A mammalian host cell useful for producing rAAV in the absence of a helper adenovirus comprising:

- (a) a transgene under the control of regulatory sequences directing expression thereof and flanked by AAV inverted terminal repeats;
- (b) an AAV *rep* sequence and an AAV *cap* sequence under the control of regulatory sequences directing expression thereof; and
- (c) adenovirus DNA sequences consisting of the minimum adenovirus DNA required to express an E1a gene product, an E1b gene product, and an E2a gene product.

2. The host cell according to claim 1 wherein said regulatory sequences directing expression of said transgene comprise a P5 promoter.

3. The host cell according to claim 1, wherein said adenovirus DNA which expresses said E1a gene product is a nucleic acid sequence comprising adenovirus DNA encoding said E1a gene product and a first promoter directing the expression of said E1a gene product.

4. The host cell according to claim 3, wherein said adenovirus DNA which expresses said E1b gene product is a nucleic acid sequence comprising adenovirus DNA encoding said E1b gene product and a second promoter directing the expression of said E1b gene product.

5. The host cell according to claim 4, wherein said adenovirus DNA which expresses said E2a gene product is a nucleic acid sequence comprising

adenovirus DNA encoding said E2a gene product and a third promoter directing the expression of said E2a gene product.

6. The host cell according to claim 5, wherein said second promoter is the native adenovirus E1b promoter.

7. The host cell according to claim 6, wherein said first promoter and said third promoter are not identical.

8. The host cell according to claim 6, wherein said first promoter and said third promoter are identical.

9. The host cell according to claim 5 wherein said third promoter is an inducible promoter.

10. The host cell according to claim 5 wherein said first promoter and said third promoter are inducible promoters.

11. The host cell according to claim 1, wherein said AAV *rep* and *cap* genes are stably integrated into the chromosomes of said host cell.

12. The host cell according to claim 1, wherein said adenovirus DNA is stably integrated into the chromosomes of said host cell.

13. The host cell according to claim 1, wherein said AAV *rep* and *cap* genes are present in said host cell as an episome.

14. The host cell according to claim 1, wherein said adenovirus DNA is present in said host cell as an episome.

15. The host cell according to claim 1 wherein said AAV *rep* sequence and said AAV *cap* sequence are present in a nucleic acid molecule comprising, from 5' to 3', a promoter, a spacer, and said *rep* and *cap* sequences.

16. A method for producing recombinant adeno-associated virus in the absence of contaminating helper virus or wild-type virus, comprising the step of culturing the host cell of any of claims 1-15.

17. The method according to claim 16, wherein said adenovirus DNA is expressed under the control of at least one inducible promoter, said method further comprising contacting said cultured host cells with at least one inducing agent, which controls the expression of said adenovirus E1a, E1b, and E2a gene products.

18. The method according to claim 16, wherein said adenovirus DNA consists of a first nucleic acid sequence encoding a first promoter and adenovirus DNA encoding said E1a and E1b gene products, and a second nucleic acid sequence encoding a second promoter and adenovirus DNA encoding said E2a gene product, wherein said first and second promoters are different inducible promoters directing the expression of each respective gene product.

19. The method according to claim 18 further comprising the steps of adding to said host cell culture a first inducing agent for inducing said first inducible promoter and a second inducing agent for inducing said second inducible promoter, whereby the ratio of expressed gene products may be varied for optimizing the production of rAAV in said host cells.

20. A method for producing recombinant adeno-associated virus in the absence of contaminating helper virus or wild-type virus, comprising the step of isolating a recombinant AAV from said host cell or host cell culture of any of claims 1-15 wherein said host cell or host cell culture expresses said transgene in the absence of contaminating helper virus or wildtype AAV.

21. The method according to claim 20, wherein said adenovirus DNA is expressed under the control of at least one inducible promoter, said method further comprising contacting said cultured host cells with at least one inducing agent, which controls the expression of said adenovirus E1a, E1b, and E2a gene products.

22. The method according to claim 20, wherein said adenovirus DNA consists of a first nucleic acid sequence encoding a first promoter and adenovirus DNA encoding said E1a and E1b gene products, and a second nucleic acid sequence encoding a second promoter and adenovirus DNA encoding said E2a gene product, wherein said first and second promoters are different inducible promoters directing the expression of each respective gene product.

23. The method according to claim 22 further comprising the steps of adding to said host cell culture a first inducing agent for inducing said first inducible promoter and a second inducing agent for inducing said second inducible promoter, whereby the ratio of expressed gene products may be varied for optimizing the production of rAAV in said host cells.